REMARKS

Claims 2, 4, 7-8, 10-11, 33-36, 39, and 41-42 were pending in the present application. Claims 4, 8, and 42 have been canceled. Claims 2, 7, 10, 33, 39 and 41 are amended herein. New claims 43 and 44 have been added. No new matter is added thereby.

All pending claims were rejected in the Final Rejection. In view of the foregoing amendments and arguments that follow, Applicants request withdrawal of all rejections upon reconsideration.

Preliminarily, Applicant would like to thank Examiner Singh for the telephonic interview conducted at 12:00 pm on March 31, 2009. Examiner Singh, the undersigned, Dr. Frank Grosveld (the inventor) and Dr. Roger Craig (Chairman of the licensee) participated in the interview. During the interview, proposed amendments, as reflected in the Listing of the Claims submitted herewith, and Bruggemann et al., *PNAS USA*, 86:6709-6713, 1989 (forwarded with the proposed amendments) were discussed, as were the differences between Applicant's invention and both the camelid and transgenic antibody preparation art in the context of enablement. In particular, Applicant emphasized the existence of extensive art whose scope encompassed the construction and regulated co-expression of immunoglobulin heavy chain transgenes with light chain transgenes in response to antigen in a B-cell specific manner. Examiner Singh stated that it appeared that the proposed amendments would overcome many of the outstanding rejections for written description and enablement, but that he would revisit whether or not any art rejections should be raised. The Examiner represented that, previously, the focus was upon the written description and enablement rejections. It was emphasized that

Applicant's claims recite that the antibodies are made in response to antigen challenge.

Before addressing the Office's outstanding objections/rejections, Applicant feels the following summary may be instructive. Riechmann & Muyldermans (reference E of record) describes the field of antigen-specific camelid heavy chain-only antibodies. Such antibodies can only be generated naturally following the injection of camelids with antigen. Camelid heavy chain-only antibodies are generated in a similar manner to regular tetrameric antibodies, in that VDJ rearrangement occurs followed by somatic hypermutation. It was not known then and is still not known now whether camelid heavy chain-only antibodies are generated in the same B-cells as regular tetrameric camelid antibodies comprising heavy and light chains, or whether camelid heavy chain-only antibodies are produced in a specialized class of camelid B-cells (see Zou et al (2005) J.Immunol. 175, 3769-3779, forwarded previously with Declaration of Louis M. Weiner, M.D., for post-filing discussion of the uncertainties regarding the process of heavy chain only antibody expression in camelids.

The following was also known at the time of filing this application.

(i) Only two subclasses of heavy chain-only antibody are present in camelid plasma (IgG2 and IgG3), and there was and still is no evidence that heavy chain-only antibodies might be produced through class switching via IgM.

(ii) In camelids, heavy chain-only antibodies use a special group of V gene segments, known as VHH gene segments (or exons), which carry definitive mutations at position 45, in the region where the heavy chain interacts with the light chain in regular tetrameric antibodies. VHH gene segments are not used in natural tetrameric camelid antibodies.

Thus, at the time of filing, there was no reliable method to derive heavy chain-only antibodies in response to antigen challenge other than the injection of camelids. Consequently, the field of heavy chain-only antibodies was limited to those derived from camelid.
The present application provides the solutions to a number of separate problems in the art.

(i) It provides a method to generate heavy chain-only antibodies in a non-human mammal other than camelid. Elimination of CH1 functionality from an immunoglobulin heavy chain transgene locus proves sufficient to permit the functional expression of heavy chain-only antibodies following antigen challenge in B-cells of a non-human mammal.

(ii) It provides a method for deriving heavy chain-only hybrid antibodies which comprise camelid VHH gene segments but otherwise are substantially derived from another species, in one instance humans. D and J gene segments and segments encoding constant effector regions devoid of CH1 functionality from other mammalian species (e.g. human) may be substituted for camelid in the transgene without affecting the productive expression of heavy chain-only antibodies in B-cells as a consequence of antigen challenge;

(iii) It provides a method for deriving a heavy chain-only antibody encoded by a transgene in a non-human mammal following antigen challenge using established hybridoma technology, an approach not possible using camelid.

Withdrawn Objections

Applicant acknowledges with appreciation the acceptance of the replacement drawing filed September 29, 2008 and the withdrawal of the objection to claims 7-8 and 10-11.

Information Disclosure Statement

The Office again states that multiple references have been cited in the specification that have not been considered because no copies have been provided. Applicant has submitted several Information Disclosure Statements in this application, and has provided copies of the non-patent literature and non-U.S. patents and publications listed therein. It is further noted that Applicant is not required to provide copies of every reference cited in the specification.

Priority

The Office alleges that the GB priority application fails to provide descriptive support for lox P sites that the Office believes are required by the present claims to effect class switching. As discussed during the interview summarized above, however, the present claims do not pertain to class switching. Regardless, lox P sites are not required to effect class switching. The Office is directed to the Lonberg et al patent it cited as pertinent – i.e., U.S. Patent No. 5,569,825, issued October 29, 1996. Isotype switching using switch regions of constant region exons is described. Applicant requests that the Office acknowledge that the claims are entitled to the filing date of the GB priority application.

Rejection Under 35 U.S.C. 112, first paragraph: Written Description

Claims 2, 4, 7-8, 10-11, 33-36, and 39 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Claims 4 and 8 have been canceled. Applicant traverses this rejection as applied to the remaining claims.

The Office's main contention was that the claims encompass functional homologues, derivatives, or fragments of the various VHH and VH exons or regions, but no homologues, derivatives, or fragments are disclosed. Without conceding the correctness of the rejection,

claim 2 has been amended to recite that the VHH exons comprise "naturally occurring" VHH coding sequences. Support for this amendment be found, *inter alia*, on page 9, lines 24-25, of the application as filed. No new matter is added thereby.

Applicant requests that this rejection be withdrawn.

Rejection Under 35 U.S.C. 112, first paragraph: Enablement

Claims 2, 4, 7-8, 10-11, 33-36, and 39 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. Claims 4 and 8 have been canceled. To the extent this rejection applies to the remaining claims, Applicant traverses.

The Office alleges that the claims, as previously amended, still read on somatic delivery. The Office noted that none of the claims required a non-human mammal whose genome comprises a VHH heavy chain locus. The claims have been amended herein to recite that the VHH locus is integrated into the genome of the non-human mammal. Support for this amendment can be found, *inter alia*, on page 27, lines 24-25, of the application as filed, wherein inserting the loci into the genome of a recipient animal is discussed.

The Office also asserts that the single heavy chain antibody requires involvement of VHH germline genes in which the interface residues are modified. The Office also argues, in reference to Janssens et al, that germ-line VHHs were chosen with hydrophilic amino acid codons at certain positions and that Applicant did not have in his possession the specific modification set forth in VHH1 or VHH2 as disclosed in Janssens. As explained during the interview, however, no modification and no choice of the naturally occurring VHH coding sequences is required. The naturally occurring VHH coding sequences have the necessary

modifications which have evolved in camelids to compensate for the absence of light chain. The claims, as amended, recite naturally occurring VHH coding sequences.

The Office also states that the Applicant did not have in his possession the specific modification capable of undergoing class switching, and that the breadth of the present invention embraces expressing chimeric loci comprising at least one Cμ and at least one of Cγ, Cα, Cδ, and Cε. Applicant again emphasizes that the present claims do not require at least one Cμ and at least one of Cγ, Cα, Cδ, and Cε. The Office is apparently confusing the present application with Application Serial No. 10/692,918.

The Office repeatedly states that neither the specification nor the prior art provide any details of the extent of the CH1 removal from the VHHDJ-Cg primary transcript. As Applicant emphasized previously, and again during the interview, it is the elimination of CH1 **functionality** which is desired. Thus, the extent of CH1 removal required is whatever results in the elimination of CH1 functionality. Methods for removing gene functionality via homologous recombination were routine to those skilled in the art at the priority date of the present application. Therefore, once the skilled person was taught that it was necessary to remove CH1 functionality to generate heavy chain-only antibodies, they would have had no difficulty in doing so. The inclusion of camelid VHH gene segments in the heavy chain locus extends what was already known in the art, i.e., that different V gene segments may be present in different combinations with different D and J gene segments in a functional immunoglobulin heavy chain transgene.

Indeed, at the time of filing of the present application, there was a wealth of literature

which describes the construction of functional immunoglobulin heavy chain minigenes and large immunoglobulin heavy chain loci and their expression as transgenes (see Brüggemann & Neuberger, Immunol. Today, 1996, 17:391-397 – reference B of record). All undergo rearrangement in a B-cell specific manner and may be used to generate antigen-specific antibodies.

As long ago as 1989, 12 years before the priority date of the present application, Bruggemann (PNAS, 1989, 86, 6709-6713 – copy forwarded previously) described the assembly of a hybrid un-rearranged immunoglobulin heavy chain gene comprising a mouse V gene segment, a human V gene segment, human and mouse D gene segments, human J gene segments, and a gene segment encoding a human mu constant effector region. Also, the necessary enhancer elements required for B-cell specific expression were present. The encoded constant region, however, included the CH1 effector region which is necessary for light chain binding and tetramer formation. This hybrid locus was expressed as a transgene and hybridomas established which produced tetrameric antibodies comprising human heavy chain and murine light chain.

The importance of Bruggemann and subsequent work by others is that together they show that hybrid loci comprising components from different mammal species and varying numbers of V, D and J gene segments are functional. Xu and Davis show that the presence of a single V gene is sufficient to obtain functional antibodies (2000, Immunity, 13, 37-45 – provided previously). Tuaillon describes the construction of a fully functional human immunoglobulin heavy chain minilocus comprising two different V genes (see Mol. Immunol., 2000, 37, 221-231

– **copy enclosed**). The presence of all D and J gene segments is preferable but not essential to the invention. The presence of one V gene segment, one D gene segment, one J gene segment, and a gene segment encoding an effector region is sufficient for rearrangement into a functional gene but will not maximize antibody diversity. Similarly, the presence of a LCR regulatory element is preferable but not essential for the expression of a heterologous immunoglobulin heavy chain locus in a B-cell specific manner (see, for example, Bruggemann, *PNAS*, 1989, and others who express normal immunoglobulin heavy chain transgenes in B-cell specific manner in the absence of an LCR).

Thus, it was known in the art how to construct functional immunoglobulin heavy chain minigenes. All of these minigenes, however, comprised the functional CH1 domains necessary for light chain binding. Applicant discovered that the absence of CH1 functionality in all constant region exons incorporated into the transgene, as claimed, ensures the formation of single heavy chain antibodies devoid of light chain in response to antigen challenge.

The application as filed provides all necessary information for one skilled in the art to practice the invention. This was **confirmed** in Janssens et al. The Office repeatedly states that Janssens et al. is a post-filing reference, ignoring Applicant's previous demonstration that Janssens el al. followed what was described in the specification or was already known in the art. Janssens et al. demonstrated the successful generation of multiple classes of human heavy chain-only antibodies from transgenic heavy chain-only antibody loci comprising camelid VHH gene segments, human D and J gene segments and gene segments encoding human constant effector regions lacking CH1 functionality. Anyone skilled in the art following the teaching in

the application as filed would have obtained the same result. At the time of filing, all related technology, such as the generation of transgenic animals, was well established as was the isolation and characterization of antibodies and antibody fragments using hybridoma and display approaches.

While agreeing that the claims do not require a quantifiable titer of antibody, the Office noted that expression of single heavy chain antibody on reduced western blot gel does not provide enabling support to one of skill in the art that the antibody could be produced in any wild type chimeric nonhuman mammal to make use of such antibody irrespective of titer. Applicant has amended the claims to remove the requirement that the antibody be isolated directly from the mammal. As indicated in new claims 43 and 44, the antibody or variable region fragment can also be isolated using hybridoma technology or phage display. Support for these amendments can be found, *inter alia*, on page 29, line 6, through page 30, line 2 (discussing phage display technology using variable region fragments), page 31, lines 5-29 (discussing hybridoma technology), and page 20, lines 14-20 (term antibody includes fragments capable of binding to a selected target) of the application as filed.

Applicant requests that this rejection be withdrawn.

Applicant reserves the right to address the art considered pertinent to Applicant's disclosure at such time as it is used in a rejection.

CONCLUSION

Applicant respectfully submits that claims 2, 7, 10-11, 33-36, and 39, 41, and 43-44 are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 665-5593, if there are any questions regarding Applicants' claimed invention.

Respectfully submitted,

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